

Enantioseparation of Penconazole, Myclobutanil and Triadimefon by Cyclodextrin-Modified Micellar Electrokinetic Chromatography

Wan Aini Wan Ibrahim*, Dadan Hermawan** and M. Marsin Sanagi

Separation Science Research Group (SSRG), Department of Chemistry, Faculty of Science,
Universiti Teknologi Malaysia, 81310 UTM, Skudai, Johor, Malaysia.

*To whom correspondence should be addressed. Email: wanaini@kimia.fs.utm.my

** Permanent address: Department of Chemistry, Universitas Jenderal Soedirman (UNSOED), Purwokerto, Jawa Tengah, Indonesia.

ABSTRACT

Simultaneous enantioseparation of three chiral triazole fungicides i.e. penconazole, myclobutanil and triadimefon by cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) under acidic condition was investigated with different HP- γ -CD concentrations as chiral selector. The three triazole fungicides have an asymmetrically substituted carbon atom respectively, and they all contain two enantiomers. In order to achieve the best enantioresolution of these triazole fungicides, the HP- γ -CD concentration range was optimized from 0 to 40 mM in 10 mM increment. The results indicate that separation of enantiomers was not achieved with concentration range of HP- γ -CD from 0 to 20 mM. Simultaneous enantioseparation was successfully achieved at 30 mM and 40 mM HP- γ -CD concentration in 25 mM phosphate buffer (pH 3.0) solution that containing 50 mM SDS, 10% methanol and 5% acetonitrile. However, enantioresolution decreased for myclobutanil and triadimefon enantiomers with HP- γ -CD concentration greater than 30 mM. In addition, an increase in the concentration of HP- γ -CD caused a significant increase in the analysis time. In light of these aspects, the optimal concentration for HP- γ -CD was decided to be 30 mM for the simultaneous chiral separation of the three triazole fungicides with resolutions (R_s) between enantiomers for penconazole, $R_s = 0.81$; myclobutanil, $R_s = 1.14$; triadimefon, $R_s = 2.09$; peak efficiencies (N) greater than 108 000 for all stereoisomers and analysis time of less than 15 min.

| Enantioseparation | Penconazole | Myclobutanil | Triadimefon | Cyclodextrin-Modified MEKC |

1. Introduction

The present paper investigates influence of hydroxypropyl- γ -cyclodextrin (HP- γ -CD) concentration on chiral separation of three chiral triazole fungicides i.e. penconazole, myclobutanil and triadimefon by cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC). The HP- γ -CD is a nonionic cyclic oligosaccharides consisting of eight glucose units and has numerous chiral recognition centers. It is a derivatized neutral cyclodextrin and widely used as chiral selector due to the water solubility and low UV absorbance. It has been used as chiral selector in the CD-MEKC methods [1-4].

In our previous work [4], the optimized CD-MEKC method for the separation of two enantiomeric pairs of propiconazole was achieved. The use of a mixture of 30 mM HP- γ -CD, 50 mM SDS and methanol-acetonitrile 10%:5% (v/v) in 25 mM phosphate buffer solution was able to separate two enantiomeric pairs of propiconazole with resolution (R_s) greater than 1.50, peak efficiencies (N) greater than 400 000 and analysis time of less than 16 min.

In this study, the effect of chiral selector concentration (HP- γ -CD) on chiral separation of the three triazole fungicides (Figure 1) by reverse mode (RM)-CD-MEKC at pH 3.0 was investigated. The three triazole fungicides have different $\log P_{ow}$ values (Table 1). The three triazole fungicides have an asymmetrically substituted carbon atom respectively, and they each contain two stereoisomers. These compounds are usually used as systemic agricultural fungicides and widely used on fruits and vegetables such as apples, cucumber, and tomato (triadimefon), artichoke, asparagus and tomato (myclobutanil), apples, grapes and tomato (penconazole). To the best of our knowledge, there is no CD-MEKC study being reported on the simultaneous enantioseparation of these three chiral triazole fungicides.

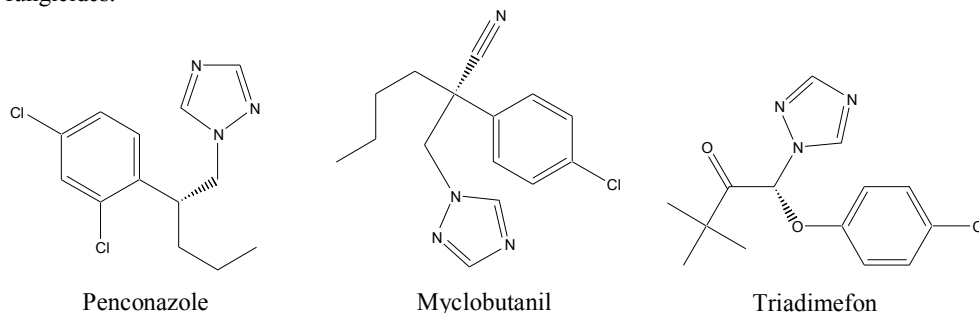


Figure 1: Structures of chiral triazole fungicides used in this study

Table 1. List of the triazole fungicides used, IUPAC names, and their $\log P_{ow}$ values

Triazole fungicides	IUPAC names	$\log P_{ow}$
Penconazole (P)	(<i>RS</i>)-1-(2,4-dichloro- β -propylphenethyl)-1 <i>H</i> -1,2,4-triazole	3.56 ^a
Myclobutanil (M)	(<i>RS</i>)-2-(4-chlorophenyl)-2-(1 <i>H</i> -1,2,4-triazol-1-ylmethyl)hexanenitrile	3.16 ^a
Triadimefon (T)	(<i>RS</i>)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1 <i>H</i> -1,2,4-triazol-1-yl)butan-2-one	2.77 ^b

^aValues taken from [5].

^bValues taken from [6].

2. Materials and Methods

All triazole fungicides were obtained from Riedel-de Haen (Seelze, Germany). The 2-hydroxypropyl- γ -cyclodextrin (HP- γ -CD) was obtained from Sigma (St. Louis, MO, USA), sodium dodecyl sulfate (SDS) was obtained from Fisher Scientific (Loughborough, UK), and disodium hydrogen phosphate 12-hydrate was obtained from Riedel-de Haen (Seelze, Germany). All other chemicals and solvents were common brands of analytical-reagent grade or better, and were used as received. Water (DD, 18 M Ω .cm) was collected from a Millipore Water Purification System (Molsheim, France).

The stock solutions of the individual triazole fungicides were prepared in methanol in the concentration range 2000 and 6000 mg/L. Final dilutions (concentrations in the figures) were prepared by diluting the stock solution with water. The separation solutions for CD-MEKC were prepared by dissolving appropriate amounts of SDS, HP- γ -CD, methanol and acetonitrile in phosphate buffers and adjusting the pH of the buffer with phosphoric acid solution. All running buffers were filtered through a 0.45 μ m nylon syringe filter from Whatman (Clifton, NJ, USA).

All electropherograms were obtained with the Agilent capillary electrophoresis system from Agilent Technologies (Waldbronn, Germany), equipped with temperature control and diode array detection (DAD). Separations were performed using an untreated fused silica capillary of 64.5 cm \times 50 μ m i.d. (with an effective length of 56-cm to the detector window) obtained from Polymicro Technologies (Phoenix, AZ, USA). Sample injection was performed hydrodynamically at 50 mbar for 1 s. The detection wavelength used was 200 nm and the capillary temperature was maintained at 20°C. The separation voltage was maintained at -30 kV (average current 40 μ A). Data were collected and processed on computer using ChemStation software (Agilent Technologies). The new capillary was conditioned by passing 1 M NaOH solution for 10 min followed by washing with deionized water for 10 min and finally equilibrating with an appropriate running buffer for 10 min. Between runs, the capillary was washed with 0.1 M NaOH, water, and run buffer for 2 min each.

3. Results and Discussion

In this study, simultaneous enantioseparation of three chiral triazole fungicides i.e. penconazole, myclobutanil and triadimefon by cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) under acidic condition was investigated with different HP- γ -CD concentrations as chiral selector. In order to achieve the best enantioresolution of these triazole fungicides, the HP- γ -CD concentration range was optimized from 0 to 40 mM in 10 mM increment. Typical enantiomeric separation of the three triazole fungicides by CD-MEKC at different HP- γ -CD concentration is shown in Figure 2. The resolutions and efficiencies obtained for triazole fungicides enantiomers at different HP- γ -CD concentrations are presented in Table 1.

The results indicate that separation of enantiomers was not achieved with concentration range of HP- γ -CD from 0 to 20 mM. Simultaneous enantioseparation was successfully achieved at 30 mM and 40 mM HP- γ -CD concentration in 25 mM phosphate buffer (pH 3.0) solution containing 50 mM SDS, 10% (v/v) methanol and 5% (v/v) acetonitrile.

It can be seen that there was no chiral resolution for all triazole fungicides at 0, 10, and 20 mM HP- γ -CD. Further increase in the concentration of HP- γ -CD up to 30 mM, increased the R_s significantly to 0.81 (for penconazole), 1.14 (for myclobutanil) and 2.09 (for triadimefon). However, further increase in the concentration of HP- γ -CD up to 40 mM, enantioresolution decreased for myclobutanil (R_s = 0.71) and for triadimefon (R_s = 1.38). While for penconazole, which is more hydrophobic than myclobutanil and triadimefon, R_s increased to 0.90. It appears that high concentration of HP- γ -CD favors the resolution of more hydrophobic triazole fungicide whereas lower concentration HP- γ -CD favors the R_s of less hydrophobic triazole fungicides. The difference of a combination of attractive forces such as hydrogen bonding, hydrophobic interactions, dipole-dipole interactions and π - π interactions between the two enantiomers and the chiral selector (cyclodextrin) plays an important role in the enantiomeric separations [7].

In addition, an increase in the concentration of HP- γ -CD caused a significant increase in the analysis time. In light of these aspects, the optimal concentration for HP- γ -CD was decided to be 30 mM for the simultaneous chiral separation of the three triazole fungicides with peak efficiencies (N) greater than 108 000 (Table 1) for all stereoisomers and analysis time of less than 15 min.

It is well-known that the principle of separation in MEKC is based on solute partitioning between the micellar phase and the aqueous/solution phase. The migration order for neutral solutes in MEKC is generally relates to the hydrophobicity of analytes [8]. In this study, separation of triazole fungicides is performed in the reverse mode MEKC (pH 3.0), which is characterized by a faster moving pseudostationary phase (PS) compared to the EOF. In acidic condition, a slower EOF occurs due to suppressed dissociation of silanol groups at the inner surface of the capillary that reduces the zeta potential. Results indicated that more hydrophobic triazole fungicide (penconazole, $\log \text{Pow} = 3.56$) interact more strongly with the micellar phase and thus migrate faster than hydrophilic compounds (myclobutanil, $\log \text{Pow} = 3.16$; triadimefon $\log \text{Pow} = 2.77$) in this reverse mode MEKC.

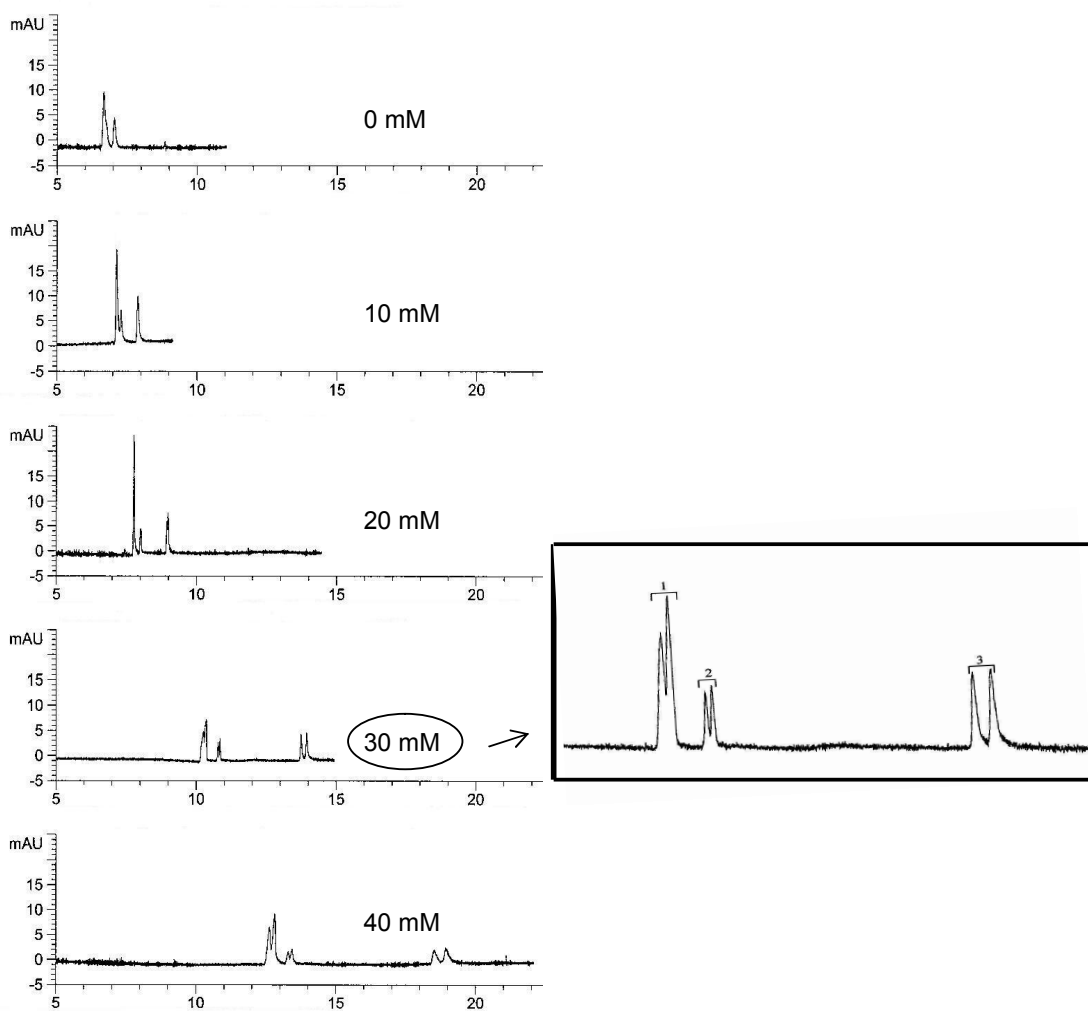


Figure 2: Separation of the three triazole fungicides by CD-MEKC with different HP- γ -CD concentrations. Separation solution: 0-40 mM HP- γ -CD, 50 mM SDS, mixed 10% methanol (v/v)-5% (v/v) acetonitrile in 25 mM phosphate buffer solution (pH 3). Sample, 300 mg L⁻¹ standards in water; injected hydrodynamically for 1 s at 50

mbar; separation voltage, -30 kV, temperature, 20 °C, detection wavelength, 200 nm. Peaks: 1. Penconazole 2. Myclobutanil 3. Triadimefon.

Table 1: Resolutions (R_s) and efficiencies (N) of triazole fungicide enantiomers by CD-MEKC with different HP- γ -CD concentrations

[HP- γ -CD] (mM)	R_s			N					
	P1-P2	M1-M2	T1-T2	P1	P2	M1	M2	T1	T2
0	0	0	0	-	-	-	-	-	-
10	0	0	0	-	-	-	-	-	-
20	0	0	0	-	-	-	-	-	-
30*	0.81	1.14	2.09	108 513	168 777	461 365	467 570	356 866	321 276
40	0.90	0.71	1.38	53 605	65 871	64 348	68 410	57 042	77 468

Analysis conditions as in Figure 2; notation:

P: Penconazole; M: Myclobutanil; T: Triadimefon.

1 and 2 are first and second-migrating peaks of triazole fungicides enantiomers, respectively.

4. Conclusions

Simultaneous enantioseparation of the three triazole fungicides was successfully achieved at 30 mM HP- γ -CD concentration in 25 mM phosphate buffer (pH 3.0) solution containing 50 mM SDS, 10% (v/v) methanol and 5% (v/v) acetonitrile with resolutions (R_s) between enantiomers for penconazole, $R_s = 0.81$; myclobutanil, $R_s = 1.14$; triadimefon, $R_s = 2.09$; peak efficiencies (N) greater than 108 000 for all stereoisomers and analysis time of less than 15 min. The optimized CD-MEKC system is simple, rapid and minimal sample requirement compared to the conventional chromatographic method [7].

5. Acknowledgement

The authors gratefully acknowledge a research grant from the Ministry of Higher Education Malaysia, for financial support through the Fundamental Research Grant Scheme (FRGS) with the grant number 78074.

6. References

- [1] Otsuka, K., Matsumura, M., Kim, J.B., Terabe, S. (2003). On-line Preconcentration and Enantioselective Separation of Triadimenol by Electrokinetic Chromatography Using Cyclodextrins as Chiral Selectors. *J. Pharm. and Biomed. Anal.* **30**. 1861-1867.
- [2] Penmetsa, K.V. (1997). *Applications of Capillary Electrophoresis in Pesticide Analysis and Toxicology Studies*. PhD Dissertation, Department of Toxicology, North Carolina State University.
- [3] Edwards, S.H., Shamsi, S.A. (2002). Chiral Separation of Polychlorinated Biphenyls Using a Combination of Hydroxypropyl- γ -Cyclodextrin and a Polymeric Chiral Surfactant. *Electrophoresis*. **23**. 1320-1327.
- [4] Wan Ibrahim, W.A., Hermawan, D., Sanagi, M.M. (2007). On-line preconcentration and Chiral Separation of Propiconazole by Cyclodextrin-Modified Micellar Electrokinetic Chromatography. *J. Chromatogr. A*. **1170**. 107-113.

- [5] Wan Ibrahim, W.A., Hermawan, D., Hasan, M.N., Aboul-Enein, H.Y., Sanagi, M.M. (2008). Rapid Estimation of Octanol-Water Partition Coefficient for Chiral Triazole Fungicides by Micellar Electrokinetic Chromatography. *Chromatographia*. **68**, 415-419.
- [6] <http://www.syrres.com/esc>. Estimation Software - KowWin Demo (checked on May 2008).
- [7] Wang, P., Jiang, S., Liu, D., Wang, P., Zhou, Z. (2005). Direct Enantiomeric Resolutions of Chiral Triazole Pepticides by High-Performance Liquid Chromatography. *J. Biochem. Biophys. Methods*. **62**, 219-230.
- [8] Nishi, H. and Terabe, S. (1996). Micellar Electrokinetic Chromatography Perspectives in Drug Analysis. *J. Chromatogr. A*. **735**, 3-27.